JACC Vol. 21, No. 3
March 1, 1993:768–76

Tetralogy of Fallot, Truncus Arteriosus, Abnormal Myocardial Architecture and Anomalies of the Aortic Arch System Induced by Bis-Diamine in Rat Fetuses

TOSHIRO KURIBAYASHI, MD,* WILLIAM C. ROBERTS, MD, FACC
Bethesda, Maryland

Objectives. The aim of this study was to analyze the relation between anomalies of the heart and aortic arch arteries in near-term rat fetuses exposed to the chemical bis-diamine.

Background. Bis-diamine is known to induce cardiovascular anomalies.

Methods. Bis-diamine was given orally to normal pregnant rats, and the 65 fetuses were examined under a dissecting microscope after formalin fixation.

Results. There were 26 rat fetuses (40%) with a ventricular septal defect in the perimembranous portion, of which 14 (22%) had tetralogy of Fallot, 4 (6%) had truncus arteriosus and 8 (12%) had a relatively small defect with no other major anomalies. In 44 fetuses (68%) the middle latitudinal muscle bundle of the ventricular septum was continuous with the right ventricular free wall. There were, isolated or in association, a double- or right aortic arch in 6 fetuses (9%), aberrant subclavian arteries in 9 (14%), right ductus arteriosus in 12 (18%) and agenetic ductus in 4 (6%). The cross-sectional area of the ductus, as corrected by that of the aortic isthmus, was abnormally small in 47 rats (72%). The rat fetuses with a septal defect or abnormal myocardial architecture, or both, usually had a small ductus; it was very small or absent in those fetuses with tetralogy of Fallot. Of the four fetuses with truncus arteriosus, two had a vestigial vasculature on the truncus root and three had a rudimentary infundibulum.

Conclusions. The cardiac defect may be the anomalous and reduced development of the sixth arch arteries, which by imposing pressure overload on the fetal right ventricle, may have led to either or both the persistence of ventricular septal defect as a vent or the formation of myocardial architecture favorable for the generation of pressure in the right ventricle.

(J Am Coll Cardiol 1993;21:768–76)

Administration of bis-diamine to pregnant rats at a critical stage produces cardiovascular anomalies in the fetuses, together with various kinds of congenital malformations of other organs such as face, forelimbs, thymus, diaphragm and kidney (1–5). The induced cardiovascular anomalies include truncus arteriosus, hypoplasia or atresia of the pulmonary trunk, a right or double-aortic arch, ventricular septal defect, common atrioventricular canal and tetralogy of Fallot. These anomalies are considered to be the model of the DiGeorge syndrome (3), III–IV pharyngeal pouch syndrome (6) or conotruncal anomaly face syndrome (7) in humans.

This teratogenic chemical was employed in the present study because the induced cardiovascular anomalies were similar to those occurring genetically in the WKY/NCrj substrain of the Wistar-Kyoto rat (8–10). We wanted to know whether the cardiac anomalies were related to those of the aortic arch system, particularly to hypoplasia of the ductus arteriosus, in the manner demonstrated in WKY/NCrj rats. Thus, we examined the abnormalities of the heart and great arteries quantitatively in a three-dimensional setting and analyzed the relation between them. In addition, we examined the abnormalities of myocardial fiber architecture; studies on WKY/NCrj rats have also suggested that these abnormalities might predispose to postnatal development of muscle fiber disarrangement similar to that seen in hypertrophic cardiomyopathy (9–12).

Methods

Study animals. We used normal 10-week old Wistar rats from Shimizu Jikken-Zairyo. They were mated overnight and pregnancy was confirmed by identifying sperm in the vaginal smear the next morning. The day of identification was considered day zero of gestation. A single dose of 200 mg of bis (dichloroacetyl) diamine (SIGMA Chemical) in a 1-ml aqueous suspension of 1% gum tragacanth was orally administered through a gastric tube to five pregnant rats in the evening of day 11 of gestation. The results were compared with those in the Wistar rat fetuses with no treatment used as a normal control group for WKY/NCrj fetuses in the previous studies (9,10), in which the sire and pregnant rats were raised under the same feeding and air conditions as in

From the Pathology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland. Dr. Kuribayashi was supported in part by Meiji Institute of Health Science, Odawara, Japan.

Manuscript received March 23, 1992; revised manuscript received July 27, 1992, accepted August 3, 1992.

*Present address and address for correspondence: Toshiro Kuribayashi, MD, Second Department of Anatomy, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji Kamigyo-Ku Kyoto, 602 Japan.

0735-1097/93/$6.00

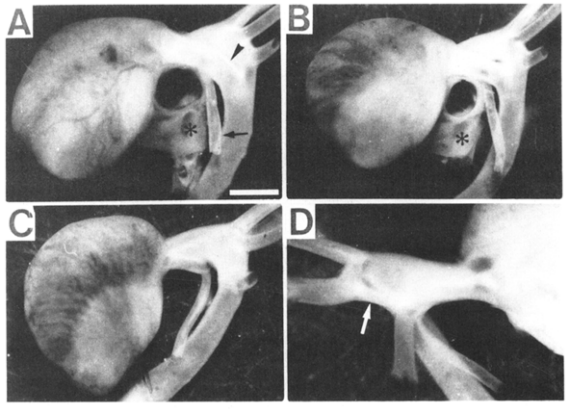

Figure 1. Heart and great vessels from fetuses on day 19. **A,** From the fetus of a nontreated rat, showing the ductus arteriosus **(arrowhead),** left pulmonary artery **(arrow)** and the left superior vena cava **(asterisk),** which is normally present in rats. Bar represents 1 mm for **A, B** and **C. B,** From the fetus of a treated rat. The ductus is small and short, whereas the aortic isthmus is large compared with that in **A. (Asterisk** as in **A.) C,** From the fetus of another treated rat. The pulmonary trunk is very small and the ductus is not visible. **D,** Right view of **C.** There is a thin right-sided ductus between the main pulmonary artery and the right subclavian artery **(arrow). In C,** the heart is rotated clockwise and its long axis is deviated toward the plane perpendicular to the descending aorta. The heart had tetralogy of Fallot (Fig. 2C).

the present study and did not produce fetuses with cardiovascular anomalies.

Protocol. With the mother rats under urethane anesthesia, 27 fetuses from two litters and 38 fetuses from three litters were removed from the uterus on day 19 and day 21 of gestation, respectively. The placenta and umbilical cord were removed simultaneously so that bleeding from the cord would not affect the size of the heart and vessels. The fetuses were then immersed in 10% formalin maintained at 18° to 20°C to fix the hearts and vessels with a minimal influence of temperature on contractile state. Fetuses obtained on day 21 were immersed in formalin after wide incision of the thorax, abdominal wall and diaphragm to fix the ductus arteriosus before its physiologic constriction (9).

The heart and great arteries were dissected out together (Fig. 1), and their gross anatomy was examined qualitatively and quantitatively under a dissecting microscope, as previously described in detail (9). The area of the ventricular septal defect and pulmonary outflow tract was determined from their major and minor diameters; the thickness of the left ventricular free wall, ventricular septum and right ventricular free wall was measured by means of an eyepiece micrometer.

The degree to which the middle latitudinal muscle bundle of the ventricular septum is continuous with the right or left ventricular free wall in the transverse plane of the heart was estimated semiquantitatively in the same way. The continuity of the septal latitudinal muscle bundle in both the ventral (anterior) and the dorsal (posterior) junctions was scored from −2 (strongly continuous with the right ventricular free wall) to +2 (strongly continuous with the left ventricular free wall), and the convexity of the septal latitudinal muscle bundle was also scored from −2 (strongly convex toward the left) to +2 (strongly convex toward the right); thus, the total score ranged from −6 to +6.

The cross-sectional area of the ductus arteriosus and the aortic isthmus at their junction was determined to estimate the flow impedance through the extracardiac outflow tracts from the right and left ventricles. We determined the ductus/aortic isthmus ratio to properly estimate the relation in size between these two structures because the area values, even if corrected by body weight raised to two thirds power, tended to be exaggerated when the body weight was small (9). At the same gestational age, the fetuses of the treated rats had a significantly smaller body weight than that of the fetuses of the nontreated rats.

Statistical analysis. Data were expressed as mean value ± SD. Individual values of experimental groups were judged to be abnormal when they were outside the mean ± 2 SD of values for the aforementioned nontreated Wistar rat fetuses (9,10). Mean values of two groups were compared by Student *t* test or Welch-Aspin *t* test (13), as appropriate. Comparison of incidence between two groups was tested by Fisher exact probability test.

Results

Hearts. A ventricular septal defect was found in 26 rat fetuses (40%) (Table 1). Right ventricular view showed it to be of the perimembranous type (Fig. 2, C to E). When viewed from the arterial side, the septal defect was subaortic, with the ventricular septum being deviated leftward and overridden by the aorta (Fig. 2F). Fourteen fetuses had a relatively large septal defect associated with an abnormally narrow pulmonary outflow tract accompanied by ventrorostrally deviated well developed muscular bands in seven

Table 1. Incidence of Abnormalities of the Heart and Aortic Arch System in Rat Fetuses Exposed to Bis-Diamine, WKY/NCrj Rat Fetuses and Patients With Thalidomide Dysmelia Syndrome

	Bis-Diamine (n = 65)		WKY/NCrj (n = 269)[a]	Thalidomide (n = 180)[b]	
	No.	%	%	No.	%
Heart					
Ventricular septal defect	26	40	18	25	14
Tetralogy of Fallot	14	22	8[c]	5	3
Truncus arteriosus	4	6	0	5	3
Double-outlet right ventricle	0	0	0	1	1
Pulmonary valve dysplasia	36	55	47	2	1
Severe[d]	8	12	17		
Mild[e]	28	43	30		
Narrow pulmonary outflow	19	30	55		
Abnormal myocardial architecture	44	68	81		
Aortic arch system					
Double- or right aortic arch	6	9	4		
Aberrant subclavian artery	9	14	0.4		
Low ductus/aorta ratio	47	72	75		
Right ductus	12	18	1.5		
Agenesis of ductus	4	6	0	4[f]	2
Pulmonary arteries					
Aortic origin	1	2	0		
Lateral wall origin	3	5	0		
Unknown nature[g]	3	5	49		
Patent ductus arteriosus or atrial septal defect				6	3

[a]Obtained from Ref. 9 and 10. Data on the heart and ductus/aorta ratio in WKY/NCrj rats are from 90 fetuses on day 21 of gestation. [b]From Ref. 14. [c]Diagnosed on the basis of ventricular septal defect with overriding aorta and severe pulmonary valve dysplasia. [d]Absence, decrease in number or severe distortion of the cusps. [e]Mild thickening and irregular cusp edges. [f]All were associated with tetralogy of Fallot. [g]A single artery diverges from the pulmonary trunk and then gives off the main right and left pulmonary arteries.

fetuses (Fig. 2, C and D) and absent bands in the other seven (Fig. 2E). Severe pulmonary valve dysplasia was associated with a large ventricular septal defect in eight fetuses (12%): agenesis of the valve was present in one fetus (Fig. 2F), the valve had one or two cusps in three fetuses (Fig. 3A) and three cusps of nonuniform size or with marked distortion were present in four fetuses. Thus, 14 fetuses with a large ventricular septal defect, overriding aorta and pulmonary or subpulmonary valve stenosis (or both) were diagnosed as having tetralogy of Fallot. Although right ventricular hypertrophy was slight in the treated fetuses (Table 2), it would be expected to progress rapidly after birth because of the drastic hemodynamic changes that occur in the presence of these anomalies (8,9).

Four rat fetuses (6%) had a large ventricular septal defect associated with truncus arteriosus (Fig. 4, Tables 1 and 3). The position and shape of the septal defect were similar to those in tetralogy of Fallot when the defect was viewed from both the right ventricle and the truncus side, with the truncus overriding the ventricular septum (Fig. 2 and 4). There was a rudiment of the right ventricular infundibulum in three hearts with truncus arteriosus (Fig. 4, D and E). The truncus arose from the left ventricle (Fig. 4C) as did the large aorta in tetralogy of Fallot (Fig. 2B); in the hearts with tetralogy or truncus, the anterior (ventral) mitral leaflet was continuous with the left and posterior (dorsal) aortic or truncal cusps, respectively (Fig. 2F, 3 and 4F) (4,5). Three fetuses with truncus had a truncal valve with three cusps; another fetus had four cusps, in which the anteriorly located cusp between the right and left cusps was obviously small (Fig. 3B). No truncal cusps appeared thickened or deformed. The coronary arteries originated normally.

The septal latitudinal muscle bundle had abnormal continuity with the right ventricular free wall in 44 (68%) of the 65 fetuses of the treated rats (Fig. 5, Table 2), although the degree of abnormal continuity did not show a clear correlation with other anomalies (Table 3).

The heart weight/body weight ratio and the foramen ovale also tended to be abnormal in the fetuses of the bis-diamine-treated rats (Tables 2 and 3).

Fourth aortic arch arteries. The aortic arch was double in four and right-sided in two fetuses (Fig. 6, A to D); one of the latter two had an aberrant left subclavian artery continuous with a left ductus. The right subclavian artery was aberrant in eight fetuses (12%); it was associated with truncus arteriosus in one, with agenesis of the ductus in two (Fig. 2, A and B) and was continuous with a right ductus in five (Fig. 6, E and F).

Sixth aortic arch arteries. The ductus arteriosus (distal portion of the sixth arch artery [17]) was abnormally small when compared with the aortic isthmus in 47 (72%) of the fetuses of the treated rats (Fig. 1, Table 1) and had a

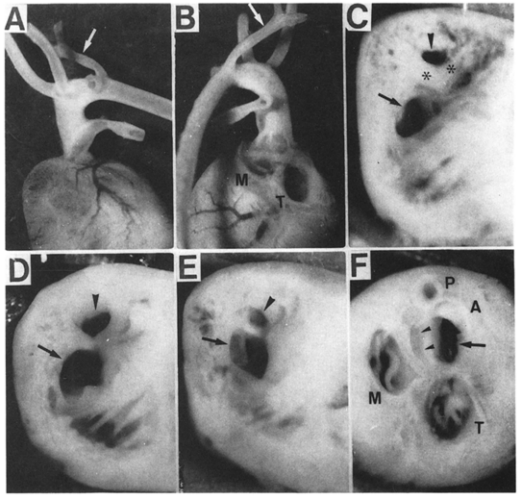

Figure 2. From fetuses with tetralogy of Fallot. **A,** The pulmonary trunk is very small, the ductus is absent and the right subclavian artery is aberrant (**arrow**). **B,** Dorsal view of **A,** showing the mitral (M) and tricuspid (T) rings. Left-sided origin of the aorta is noted. The **arrow** corresponds to that in **A. C,** Right ventricular view from the same heart shown in Figure 1C. There is a large ventricular septal defect (**arrow**) and the infundibulum (**arrowhead**) is narrow because of the development of ventrorostrally deviated parietal and septal bands (**asterisks**). **D,** From a heart with no ductus obtained on day 21. Aortic cusps on the opposite side of the defect were dissected. **Arrow and arrowhead** as in **C. E,** From the heart in **A.** There is a large ventricular septal defect and the infundibulum is very narrow. The crista supraventricularis is hypoplastic and muscular bands are not developed. **Arrow and arrowhead** as in **C. F,** Arterial view of the same heart as in D. The aorta overrides the ventricular septum, which deviated leftward. The pulmonary ring is very small and the valve cusps are absent. A, M, P, and T = aortic, pulmonary, mitral and tricuspid rings, respectively; **arrow** = ventricular septal defect; **arrowheads** = left ventricular outflow tract.

significantly smaller mean value in the fetuses of the treated rats than in the fetuses of the nontreated rats (Table 2). The ductus was right-sided in 12 rats (18%); it was continuous with a right aortic arch in 5 (Fig. 6, A to C), with the right subclavian artery in 2 (Fig. 1, C and D) and with an aberrant right subclavian artery in 5 (Fig. 6, E and F). The right-sided ductus tended to be very small, particularly when it was continuous with a right subclavian artery (Table 3). Bilateral ductus was not seen. Apart from fetuses with truncus arteriosus, the ductus was absent on both sides in four fetuses (Fig. 2A). The pulmonary trunk and ring were markedly hypoplastic in seven fetuses (11%), and each also had a markedly hypoplastic or agenetic ductus, a large aorta and tetralogy of Fallot (Fig. 1C and 2A). The hypoplastic pulmonary trunk was consistently continuous with a hypoplastic right ventricular infundibulum (Fig. 2, C to E), and it had no communication with the ascending aorta.

Four rats had truncus arteriosus with a left aortic arch. In

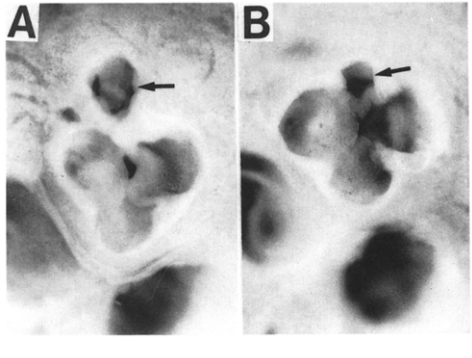

Figure 3. A, Arterial view of a heart with tetralogy of Fallot. **Arrow** identifies a small pulmonary ring with a unicuspid pulmonary valve. **B,** From a heart with truncus arteriosus. The truncal valve consists of four cusps, with the ventral cusp being obviously small (**arrow**). It has been suggested (16,18) that a quadricuspid truncal valve consists of three aortic leaflets plus a pulmonary leaflet remnant.

JACC Vol. 21, No. 3
March 1, 1993:768–76

Table 2. Quantification of the Heart and Aortic Arch Arteries

	Fetuses on Day 19		Fetuses on Day 21	
	Mother Not Treated (n = 24)	Mother Treated (n = 27)	Mother Not Treated (n = 28)	Mother Treated (n = 38)
Body weight (g)	3.15 ± 0.17	2.55 ± 0.30* (81%)	5.16 ± 0.52	4.52 ± 0.54* (29%)
Heart weight/body weight ratio (mg/g)	4.97 ± 0.32	5.05 ± 0.55 (7%)	5.24 ± 0.39	5.92 ± 0.58* (37%)
Ventricular septum/left ventricular wall ratio	1.44 ± 0.10	1.55 ± 0.12*	1.37 ± 0.12	1.40 ± 0.16
Right/left ventricular free wall thickness ratio	NM	NM	0.90 ± 0.10	0.99 ± 0.09*
Pulmonary outflow tract ($\times 10^3$ μm^2)	152 ± 25	122 ± 40* (26%)	144 ± 21	112 ± 55* (32%)
Continuity of septal latitudinal muscle bundle	1.8 ± 0.8	0.2 ± 1.1* ≤0 (63%)	1.5 ± 0.7	0.1 ± 1.3* ≤0 (71%)
Foramen ovale ($\times 10^3$ μm^2)	154 ± 23	209 ± 59* (52%)	69 ± 22	121 ± 45* (50%)
Ductus arteriosus ($\times 10^3$ μm^2)	49 ± 5	36 ± 11* (44%)	41 ± 4	31 ± 17* (37%)
Aortic isthmus ($\times 10^3$ μm^2)	29 ± 3	49 ± 19* (-%)	23 ± 3	43 ± 18* (76%)
Ductus/aorta ratio	1.69 ± 0.18	0.90 ± 0.43* (85%)	1.83 ± 0.21	0.93 ± 0.54* (84%)

*$p < 0.01$ in comparison with fetuses in nontreated groups. Data are expressed as mean value ± SD. Area values were corrected by body weight (g) raised to the power of two thirds. The value of the pulmonary outflow tract and ductus arteriosus was considered to be zero in cases of truncus arteriosus. Fetuses from nontreated mothers were examined in previous studies (9,10) as a control group for WKY/NCrj rat fetuses. NM = not measured; % = percent of rat fetuses with abnormal values.

three, a single artery originated from the truncus and then gave off the right and left main pulmonary arteries (Fig. 4A), a type consistent with *type I persistent truncus arteriosus* in humans according to the classification of Collett and Edwards (15) or *type I truncus arteriosus (communis)* according to the classification of Van Praagh and Van Praagh (16,18).

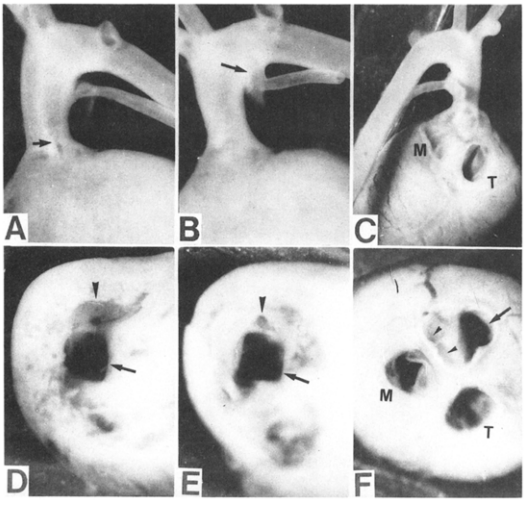

Figure 4. From fetuses with truncus arteriosus. **A,** A single pulmonary trunk arises from the truncus and then gives off the right and left main pulmonary arteries. There is a vestige of vasculature in the truncus wall between the right and left sinuses **(arrow). B,** The main pulmonary arteries are continuous with the truncus through a small left-sided ductus arteriosus **(arrow). C,** Posterior view of **A.** The origin of the truncus is left-sided as the aorta from the heart with tetralogy in Figure 2B. **D** and **E,** Right ventricular view of the hearts with truncus arteriosus. **Arrow** = ventricular septal defect with truncal cusps on the opposite side. In both hearts, there is a structure suggestive of a rudiment or remnant of the infundibulum **(arrowhead). F,** Arterial view with the cusps dissected. **Arrow** = ventricular septal defect; **arrowheads** = left ventricular outflow tract. There is no pulmonary ring. The truncus overrides the ventricular septum as does the aorta in the heart with tetralogy in Figure 2F. Abbreviations as in Figure 2.

Table 3. Relations Among Anomalies of the Aortic Arch Arteries and the Heart

		Aortic Arch (AA) Anomalies					Ventricular Septal Defect	
	Total (n = 65)	No AA Anomaly (n = 44)	Double or Right AA (n = 6)	Right DA (n = 7)	Agenesis of DA (n = 4)	TA (n = 4)	Absent (n = 39)	Present (n = 26)
Ductus arteriosus ($\times 10^3$ μm^2)		41 ± 5	37 ± 7	20 ± 12	0	0	41 ± 5	21 ± 18*
	(25)	[7]	[4]	[6]	[4]	[4]	[8]	[17‡]†
Aortic isthmus ($\times 10^3$ μm^2)		37 ± 8	41 ± 19	73 ± 18	75 ± 3	77 ± 7	36 ± 7	62 ± 20*
	(49)	[29]	[5]	[7]	[4]	[4]	[24]	[25]‡
Ductus/aorta ratio		1.16 ± 0.25	1.05 ± 0.41	0.31 ± 0.26	0	0	1.20 ± 0.25	0.45 ± 0.45*
	(55)	[35]	[5]	[7]	[4]	[4]	[30]	[25]‡
Ventricular septal defect ($\times 10^3$ μm^2)	(26)	1–41 [8]	6–45 [3]	6–103 [7]	123–156 [4]	*55–179 [4]		
Pulmonary outflow tract ($\times 10^3$ μm^2)	(20)	141 ± 26 [4]‡	135 ± 29 [2]	68 ± 29 [6]	39 ± 12 [4]	0 [4]	142 ± 25 [2]	71 ± 49* [18]†
Continuity of septal latitudinal muscle bundle	(44)	0.3 ± 1.1 [31]‡	0.5 ± 0.8 [2]	−0.7 ± 1.3 [6]	−0.5 ± 1.1 [3]	0 ± 0.8 [2]	0.3 ± 1.1 [26]	−0.2 ± 1.2 [18]
Foramen ovale ($\times 10^3$ μm^2)§	(26)	110 ± 39 [11]‡	60–129 [2]	139–147 [5]	141 ± 33 [4]	195 ± 54 [4]	111 ± 40 [16]	142 ± 51* [10]
Heart weight/body weight ratio (mg/g)§	(19)	5.65 ± 0.43 [4]	6.01–6.20 [2]	6.50–6.66 [5]	5.87 ± 0.06 [4]	6.54 ± 0.34 [4]	5.67 ± 0.44 [6]	6.44 ± 0.48* [13]†

*$p < 0.01$, †$p < 0.05$ in comparison with groups with no ventricular septal defects. ‡One fetus with tetralogy that had a ductus of normal size is not included here; the left pulmonary artery diverged from the aorta in this rat (Fig. 6A). §Mean values were obtained only from rats on day 21 of gestation because these values differ greatly between rats on day 21 and day 19. Values without parentheses or brackets are expressed as mean value ± SD or range of values; values were expressed as a range when the number of fetuses with the lesion in the column shown was <3. Values in parentheses indicate the number of fetuses with the abnormality shown in column 1. Values in brackets show the number of fetuses with both the abnormality in column 1 and the abnormality in the column shown. The values of the pulmonary outflow tract and ductus arteriosus were considered to be zero when truncus arteriosus was present. Five of the six fetuses of the Double or Right AA group had a right ductus continuous with the right aortic arch. All ductus in the Right DA group were continuous with the right subclavian artery. DA = ductus arteriosus; TA = truncus arteriosus.

Although the pulmonary components in these fetuses were very small compared with the size of the truncus, these components would be expected to become larger soon after birth when pulmonary flow increases rapidly, as shown previously (9). In the remaining fetus, the main pulmonary arteries were continuous with the truncus by way of a hypoplastic left ductus (Fig. 4B), a type considered to be due not to the failure of truncal septation but to agenesis of the

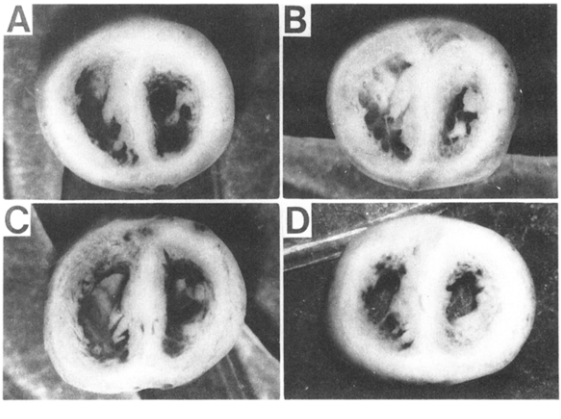

Figure 5. Transverse midventricular cut surface of the hearts from fetuses of bis-diamine–treated rats on day 21. **A,** From a fetus with a normal-sized ductus arteriosus. **B,** From a fetus with a small ductus and a large aorta but with no ventricular septal defect. **C,** From a heart with tetralogy of Fallot. **D,** From a heart with truncus arteriosus. In A and D, the middle latitudinal muscle bundle is convex toward the right and continuous with the left ventricular free wall, whereas in B and C it is straight or convex toward the left and continuous with the right ventricular free wall. The continuity index in A, B, C and D was estimated to be 3, −3, −1 and 2, respectively.

JACC Vol. 21, No. 3
March 1, 1993:768–76

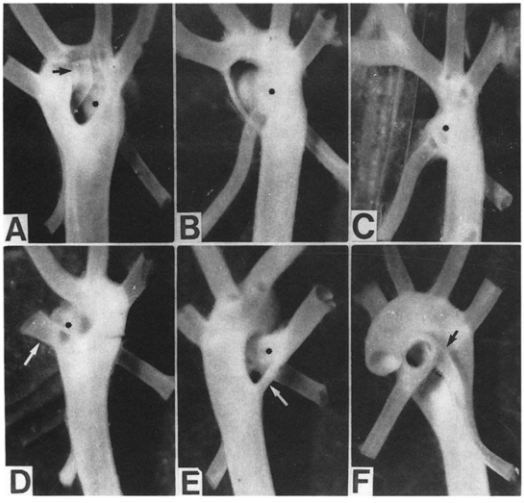

Figure 6. Various aspects of aortic arch anomaly. **Asterisks** identify the ductus arteriosus. **A,** Dorsal view of a double-aortic arch with a right ductus. **Arrow** shows anomalous origin of the left main pulmonary artery from the aortic arch. **B,** Dorsal view of a double-aortic arch with marked hypoplasia of the left arch. **C,** Dorsal view of a right aortic arch with a right ductus. Mirror image branching is noted; the first branch is the left brachiocephalic artery. **D,** Dorsal view of a right aortic arch with a left ductus that is continuous with an aberrant left subclavian artery **(arrow)**, producing a vascular ring. **E,** Dorsal view of a vascular ring due to a left aortic arch and a right ductus that is continuous with an aberrant right subclavian artery **(arrow)**. The proximal portion of the subclavian artery is markedly hypoplastic, suggesting a developmental process of its isolation. **F,** Ventral view of a vascular ring due to a right ductus and an aberrant right subclavian artery. The right main pulmonary artery normally diverges caudally from the pulmonary trunk, whereas the left main pulmonary artery abnormally arises from the left lateral side **(arrow)**.

pulmonary trunk and hence termed pseudotruncus arteriosus (15,19). There was a vestigial vasculature on the truncus root between the right and left sinuses in two fetuses (Fig. 4A).

The left main pulmonary artery originated from the caudal side of aortic arch segment in one fetus (Fig. 6A) and from the left lateral side of the pulmonary trunk in three (Fig. 6F); three others had a single artery that diverged caudally from the pulmonary trunk and then gave off the right and left main pulmonary arteries, just as the pulmonary components diverged from the truncus arteriosus shown in Fig. 4A.

Whether it was an isolated abnormality or was associated with other aortic arch anomalies, the ductus arteriosus was small when the fetus had ventricular septal defect, a narrow pulmonary outflow tract, pulmonary valve dysplasia, a wide foramen ovale or abnormal myocardial architecture (Table 3); in particular, the ductus was very small or absent when tetralogy of Fallot was present, except in one fetus whose left main pulmonary artery diverged from the aortic arch (Fig. 6A).

Discussion

Although fetuses from the same litter must have been exposed to the same teratogen at the same time, various cardiovascular anomalies developed in manifold combination in these fetuses. The alternative explanation is that there were multiple points of action or that a simple common process underlay their development. The vulnerable period is from day 8 to day 12 of gestation (1–5), which just precedes or partly overlaps the following process. Orifice pairs of the fourth and sixth arch arteries appear in order in the aortic sac on day 11, the sixth pair fusing soon thereafter (2,17). The fusion of the ridges in the aortic sac and the *embryonic truncus arteriosus* leads to the aorticopulmonary septation before day 13; the pulmonary side and the already fused pair of the proximal portion of the sixth arch artery form the pulmonary trunk and ring (2). The conal septation is then completed 1 day later (20). In previous studies (1 to 5), the treatment of mother rats on days 8 to 11 caused a 20% to 100% incidence of truncus arteriosus; the much lower incidence of truncus in the current study may be due to a later treatment on day 11.5.

We first clarified quantitatively in the rat fetuses exposed to bis-diamine that there was a serial variation of anomalies of the ductus arteriosus, ranging from mild hypoplasia and right-sidedness to agenesis. This spectrum may include the pulmonary trunk and ring. The truncus arteriosus with pulmonary supply solely by way of a ductus has been suggested to be caused by disappearance of the proximal portion of the sixth aortic arch arteries that was once present (15,19). Moreover, if we suppose that a similar developmental arrest and subsequent regression occurred in the proximal portion of the sixth arch arteries with no distal portion (see Fig. 2A), we can easily imagine that the result would be so-called type I truncus arteriosus. The vestigial vasculature

on the truncus root (Fig. 4A) seems to support this speculation.

Thus, the truncus arteriosus in this study may lie on the extreme side of the spectrum of reduced and anomalous development of the sixth arch arteries, although this view theorerically does not exclude the possibility that the truncal septation may not have occurred at the same time. Van Mierop et al. (21) referred to a possible association of the failure of truncal septation with absence or early involution of the sixth arch arteries. Rychter (22) concluded from his experimental results that mesenchyma located in the anlagen of the sixth arch arteries may participate in the truncal septation. Okamoto et al. (2) reported that in all fetuses exposed to bis-diamine during days 9 and 10, the position and shape of the sixth arch orifice were definitely abnormal and the truncal ridges were hypoplastic with no fusion when observed during days 12 to 14 with a scanning electron microscope.

Tetralogy of Fallot is often associated with anomalies of the aortic arch system in humans (23), dogs (24), chicks (25) and rats (9). In the present rat fetuses, tetralogy was almost always associated with a small or absent ductus, sometimes together with a right or a double-aortic arch, showing a close resemblance to that seen in WKY/NCrj rats (9) (Table 1). An effect of hemodynamic status in cardiogenesis has long been suspected (26). In this regard, the previous study on WKY/NCrj rats (9) has suggested that perimembranous ventricular septal defect may persist as a vent of high pressure from the right ventricle to the aorta due to an increased impedance through a small ductus, thereby leading to a leftward deviation of the ventricular septum with overriding of the aorta, narrowing of the pulmonary outflow tract and enlargement of the aortic isthmus. This speculation is supported by experimental results in which mechanical intervention of the extracardiac (27,28) or intracardiac (29) pulmonary outflow produced a subaortic ventricular septal defect with a right to left shunt in the late fetal stage and intervention of extracardiac left ventricular outflow resulted in persistence of a subpulmonary ventricular septal defect with a left to right shunt (27,30).

The appearance of the ventricular septal defect from both the right ventricle and the arterial side was similar in fetuses with tetralogy of Fallot and truncus arteriosus; in addition, three of four fetuses with truncus had a rudiment or remnant of the right ventricular infundibulum. This, together with the vascular vestige on the truncus root, supports the view that tetralogy and truncus are closely related and that truncus is an extreme form of tetralogy. The anatomic resemblance of the two anomalies was pointed out by Van Praagh and Van Praagh in human cases (16,18). The occurrence of truncus in a dog strain (21) that, like the WKY/NCrj rat strain, develops tetralogy of Fallot genetically (24) also supports the presence of a close relation between the two anomalies. Truncus and tetralogy were predominant cardiovascular anomalies in human patients with the thalidomide dysmelia syndrome (14) (Table 1).

The myocardial abnormality, in which the septal latitudinal muscle bundle was continuous with the right ventricular free wall, also was seen in WKY/NCrj rat hearts (Table 1) (9). This myocardial architecture, which favors the generation of pressure in the right ventricle, may have occurred as a result of pressure overload on the right ventricle caused by a small ductus, although the presence of a large ventricular septal defect as a vent may have modified the hemodynamic effect. The abnormal rightward continuity should be inadequate for the generation of pressure in the left ventricle after birth. However, once the abnormality is established before birth, its resolution may be difficult, because cardiac myocytes lose their proliferating capacity rapidly after birth (31). This condition may lead to the postnatal development of myocardial disarray often present in hypertrophic cardiomyopathy, as suggested in the studies on WKY/NCrj rats (8–12). Morphologic analysis of human autopsy hearts with the disease (32) strongly suggested that this speculation may be applicable in many human cases as well.

We have suggested that the most important defect common to the present rat fetuses is reduced development of the sixth aortic arch arteries, with their hemodynamic consequences leading to various cardiac anomalies. This speculation, however, depends solely on morphology in the present bis-diamine–treated Wistar rats and WKY/NCrj rats (9); fetal hemodynamic studies (33,34) may allow us to approach functional aspects of fetal morphologic findings. There is evidence that the neural crest cells migrate to the aortic arch system including sixth arch arteries (35). Ablation of the neural crest from the chick embryo frequently results in anomalies of the heart and aortic arch system (36,37). Nimustine hydrochloride has a cytotoxic effect on the neural crest cells and also produces similar anomalies (38). Thus, the anomalous development in the present rat fetuses as well as WKY/NCrj rats may also be related to an impediment of the neural crest including a possible disorder of its cell migration; however, it is unclear why the sixth arch arteries are most susceptible in both models.

There were some differences between the two models. In the present rat fetuses, the pulmonary trunk and ring were obviously hypoplastic when the ductus was small or absent, whereas they were normal-sized in WKY/NCrj rat fetuses even when the ductus was very small (9). It has been demonstrated (17) that the ductus on each side does not develop as the extension of the proximal portion of the sixth arch artery but grows directly from the dorsal aorta and then fuses with the proximal portion and the main pulmonary artery, which are growing synchronously. Thus, the ductus and pulmonary trunk may possibly grow under different genetic conditions, explaining their discrepant development in WKY/NCrj rats. In the present rats, however, the two portions growing in parallel should have been exposed to the teratogen at the same vulnerable phase, resulting in the concurrence of their reduced development. In the seven rats with a very small pulmonary trunk and ring, the pulmonary valve cusps were agenetic, reduced in number or of nonuni-

form size. This is an additional feature not observed in WKY/NCrj fetuses in which severe dysplasia of the cusps consisted of marked thickening, distortion or commissural fusion due possibly to augmented mechanical stimulus to closure of the cusps (9). There may be a different developmental process for the severe pulmonary valve dysplasia observed in the present rats.

We are grateful to the staff of the Pathology Branch of the National Heart, Lung, and Blood Institute, National Institutes of Health for providing facilities for this study.

References

1. Taresposos P, Salgo MP, Oster G. Teratogenic action of a bis (dichloroacetyl) diamine on rats: patterns of malformations induced in high incidence at time-limited periods of development. Teratology 1978;18:5–16.
2. Okamoto N, Satow Y, Lee JA, et al. Morphology and pathogenesis of the cardiovascular anomalies induced by bis-(dichloroacetyl)-diamine in rats. In: Nora JJ, Takao A, eds. Congenital Heart Disease: Causes and Processes. Mt Kisco, NY: Futura, 1984:199–221.
3. Ikeda T, Matsuo T, Kawamoto K, Iwasaki K, Jubashi T. Bis-diamine-induced defects of the branchial apparatus in rats. In Ref 2: 223–35.
4. Momma K, Ando M, Takao A. Fetal cardiac morphology of tetralogy of Fallot with absent pulmonary valve in the rat. Circulation 1990;82:1345–51.
5. Momma K, Ando M, Takao A, Tomita S. Fetal cardiovascular morphology of truncus arteriosus with or without truncal valve insufficiency in the rat. Circulation 1991;83:2094–100.
6. Freedom RM, Rosen FS, Nadas AS. Congenital cardiovascular disease and anomalies of the third and fourth pharyngeal pouch. Circulation 1972;56:165–72.
7. Shimizu T, Takao A, Ando M, Hirayama A. Conotruncal anomaly face syndrome: its heterogeneity and association with thymus involution. In Ref 2: 21–41.
8. Kuribayashi T, Shimoo K, Nakamura T, et al. Tetralogy of Fallot, pulmonary valve stenosis, ventricular septal defect, and hypertrophic cardiomyopathy in WKY/NCrj rats. Pediatr Res 1990;27:483–7.
9. Kuribayashi T, Shimoo K, Nakamura T, et al. Tetralogy of Fallot, cardiac hypertrophy, pulmonary hyperte.sion, and anomalies of great vessels in fetuses and neonates of WKY/NCrj rats. Pediatr Res 1990;28:429–36.
10. Kuribayashi T, Tanawaki M, Nakanichi K, et al. Hereditary transmission of tetralogy of Fallot, cardiac hypertrophy, and anomalies of great vessels in WKY/NCrj rats. Pediatr Res 1991;30:227–31.
11. Kuribayashi T. Spontaneously occurring hypertrophic cardiomyopathy in the rat. I. Pathologic features. Jpn Circ J 1987;51:573–88.
12. Kuribayashi T, Mizuta T, Shimoo K, et al. Spontaneously occurring hypertrophic cardiomyopathy in the rat. II. Distribution of, and correlations between, various cardiac abnormalities in the WKY/NCrj and its related strains. Jpn Circ J 1988;52:1156–70.
13. Welch BL, Aspin AA. Tables for use in comparisons whose accuracy involves two variances, separately estimated. Biometrika 1949;36:290–6.
14. Keck EW, Roloff D, Markworth P. Cardiovascular findings in children with the thalidomide dysmelia syndrome. Proc Assoc Eur Paediatr Cardiol 1972;8:66–9.
15. Collett RW, Edwards JE. Persistent truncus arteriosus: a classification according to anatomic types. Surg Clin North Am 1949;29:1245–70.
16. Van Praagh R, Van Praagh S. The anatomy of common aorticopulmonary trunk (truncus arteriosus communis) and its embryologic implications. A study of 57 necropsy cases. Am J Cardiol 1965;16:406–25.
17. de Ruiter MC, Gittenberger-de Groot AC, Rammos S, Poelmann RE. The special status of the pulmonary arch artery in the branchial arch system of the rat. Anat Embryol 1989;179:319–25.
18. Van Praagh R. Truncus arteriosus: what is it really and how should it be classified. Eur J Cardiothorac Surg 1987;1:65–70.
19. Sotomora RH, Edwards JE. Anatomic identification of so-called absent pulmonary artery. Circulation 1978;57:624–33.
20. Vuillemin M, Pexieder T. Normal stage of cardiac organogenesis in the mouse. II. Development of the internal relief of the heart. Am J Anat 1989;184:114–28.
21. Van Mierop LHS, Patterson DF, Schnarr WR. Pathogenesis of persistent truncus arteriosus in light of observations made in a dog embryo with the anomaly. Am J Cardiol 1978;41:755–62.
22. Rychter Z. Analysis of relation between aortic arches and aorticopulmonary septation. Birth Defects: Original Article Series 1978;14:443–8.
23. Satyanarayana Rao BN, Anderson RC, Edwards JE. Anatomic variation in the tetralogy of Fallot. Am Heart J 1971;81:361–71.
24. Van Mierop LHS, Patterson DF, Schnarr WR. Hereditary conotruncal septal defect in Keeshond dogs: embryologic studies. Am J Cardiol 1977;40:936–50.
25. Hodach RJ, Gilbert EF, Faloon JF. Aortic arch anomalies associated with the administration of epinephrine in chick embryos. Teratology 1974;9:203–10.
26. Jaffee OC. Hemodynamics and cardiogenesis. The effect of physiologic factors on cardiac development. Birth Defects: Original Article Series 1978;14:393–404.
27. Rychter Z. Experimental morphology of the aortic arches and the heart loop in chick embryos. Adv Morphogen 1962;2:333–71.
28. Clark EB, Martini RD, Rosenquist DC. Effect of lung bud excision on cardiopulmonary development in the chick. Birth Defects: Original Article Series 1978;14:423–9.
29. Aranega A, Egea J, Alvarez L, Arteaga M. Tetralogy of Fallot produced by mechanical interference with cardiogenesis. Anat Rec 1985;213:560–5.
30. Jaffee OC. Hemodynamic factors in the development of the chick embryo heart. Anat Rec 1965;151:69–75.
31. Zac R. Development and proliferative capacity of cardiac muscle cells. Circ Res 1976;34–35(suppl II):II-17–26.
32. Kuribayashi T, Roberts WC. Myocardial disarray at junction of the ventricular septum and left and right ventricular free walls in hypertrophic cardiomyopathy. Am J Cardiol 1992;70:1333–40.
33. Tomita H, Connuck DM, Leatherbury L, Kirby ML. Relation of early hemodynamic changes to final cardiac phenotype and survival after neural crest ablation in chick embryos. Circulation 1991;84:1289–95.
34. Nakazawa M, Miyagawa S, Morishima M, et al. Hemodynamic effects of cardiovascular agents on the embryonic circulation: a comparative study in chick and rat embryos. In: Clark EB, Takao A, eds. Developmental Cardiology: Morphogenesis and Function. Mt Kisco, NY: Futura 1990:315–23.
35. Miyagawa-Tomita S, Waldo K, Tomita H, Kirby ML. Temporospatial study of the migration and distribution of cardiac neural crest in quail-chick chimeras. Am J Anat 1991;192:79–88.
36. Kirby ML, Waldo KL. Role of neural crest in congenital heart disease. Circulation 1990;82:332–40.
37. Bockman DE, Redmond ME, Waldok K, Davis H, Kirby ML. Effects of neural crest ablation on development of the heart and aortic arch arteries in the chick. Am J Anat 1987;180:332–41.
38. Miyagawa S, Kirby ML. Pathogenesis of persistent truncus arteriosus induced by nimustine hydrochloride in chick embryos. Teratology 1989;39:287–94.